NEW SPECIES OF ACHLYA AND OF SAPROLEGNIA[1]

A. J. PIETERS

(WITH PLATE XXI)

In the course of physiological work on *Saprolegnia* carried on during the past four years, there was occasion to make pure cultures of a large number of water molds. Altogether some 85 numbers were isolated and cultivated on flies or in artificial media or both. Among these forms were several that could not be induced to produce oogonia and which could not, therefore, be referred to any species. The author is of the opinion that in some cases such forms have completely lost the power to produce oogonia and that they should be described and named as new species, the diagnoses to rest on physiological rather than on morphological bases. However, his experience with some of these forms shows that it will be well to be cautious in drawing conclusions. In the case of a variety of *Saprolegnia monoica* to be described, the form was in cultivation for 16 months, on flies and in various media, without any sign of oogonia being produced. Later, when just the right combination of conditions was presented, oogonia were produced.

Another species, to be described as *S. Kaufmanniana*, produced oogonia sparingly on flies, many cultures not showing a single oogonium. In this form also a number of oogonia with oospores and antheridia were produced in a certain strength of haemoglobin solution, but in no other medium. These experiences show that the production of sexual organs may depend on some special combination of conditions, differing doubtless for each form. One form, which has been studied for 18 months as no. 66 and which has been tested in every way in which any of the other forms have been tested, still refuses to produce oogonia, though yielding an abundant harvest of round single gemmae. These gemmae have the shape and size of oogonia, and are commonly borne laterally on short stalks just as oogonia are in such a species as *S. monoica*. In

[1] Contribution from the Botanical Laboratory of the University of Michigan, no. 148.

fact, when these gemmae first form, the observer is certain that young oogonia are being produced, but in no case have oospores or antheridia been observed, though hundreds of cultures have been examined. It is possible that in this form these gemmae are nothing but arrested oogonia, and that, could we find the proper conditions, oospores and perhaps antheridia would be developed.

Certain details in regard to these and other tests will be presented in another paper; at present the writer wishes to describe a species of *Achlya*, which he has named *A. Klebsiana* in honor of Professor GEORG KLEBS. The naming of this species for Professor KLEBS seemed to the writer especially appropriate since at one time it seemed to be an exception to the rule laid down by KLEBS in 1899 that sporangia are formed only when the food supply is quickly and markedly decreased. Further study, however, showed that this apparent exception was a real and interesting proof of the correctness of KLEBS's statement. The experiments showed that while an abundance of food was present in the solution in which sporangia were formed, it was not available to the growing hyphae and consequently might as well not have been there. While working in Heidelberg the writer observed that a culture of *A. DeBaryana* Humphrey regularly produced sporangia in agar to which pea broth had been added. The sporangia were mostly borne on the large, vigorous hyphae that made a rapid growth immediately after a fresh plate of pea agar was inoculated. Later very many slender hyphae, which grew more slowly, were formed, and these did not produce sporangia. On the strong hyphae there were often 2 or 3 sets of sporangia, but always after a time the development of sporangia ceased and the outer portions of the medium became filled with many slender hyphae. One other form which never produced oogonia behaved in a similar manner. The agar here contained an abundance of food, and yet immediately after growth commenced sporangia were formed; while later, when the amount of food present might be thought to have been decreased, no sporangia were formed. At that time no definite experiments were undertaken to explain this phenomenon. Later these cultures were lost, and it was not until the fall of 1913 that another form showing this characteristic was collected. Meanwhile, *A. prolifera*, *A. race-*

mosa, and a number of species of *Saprolegnia* had been cultivated, but none of these produced sporangia in 1.5 per cent agar with pea broth. In the fall of 1913 nos. 67, 68, and 70 were isolated, and in every case the germ tube gave rise to several vigorous hyphae which produced sporangia after attaining a length of some 5 mm.

It was also found that sporangia were sometimes developed on mycelia growing in liquid pea broth. Since there certainly was no question here of an abundant supply of food, the thought suggested itself that KLEBS's conclusion, that sporangium formation takes place only when there is a dearth of food, would not apply to all species. However, when mycelia were transferred to purified water, sporangia were normally produced.

The hypothesis suggested to explain the facts was that although there was plenty of food it did not reach the surface of the growing hyphae rapidly enough; these were therefore soon in an environment poor in food and then sporangia were normally and inevitably produced.

In the pea broth the proteid constituents are colloids, and these large molecules diffuse with extreme slowness, so slowly, in fact, that their movement can be considered to be practically nil. The large, vigorous hyphae which require a relatively large amount of food would therefore after a time find themselves surrounded by a film of liquid out of which they had absorbed the food particles and to which diffusion did not carry new particles as rapidly as the hyphae used them. This condition would result in starvation and the development of sporangia.

To test this hypothesis several series of experiments were prepared as follows. Four Ehrlenmeyer flasks, each holding 100 cc., were half filled with pea broth, sterilized, and inoculated with *A. Klebsiana*. One of these was so hung by a stout string that an arm clamped to a shaft and protected by a cotton pad pushed the flask aside about once a minute. As the flask was pushed aside, it fell against the cotton pad on the end of the arm and thus the liquid was vigorously jarred. Another flask was placed upon a shelf subject to a slight jar from a small motor, so that the surface of the liquid could be seen to tremble slightly; the other flasks were placed upon the writer's desk.

In every case where these flasks were arranged in the afternoon and examined in the morning so that those on the desk would be unaffected by the jar caused by walking, which complicated matters during the daytime, it was found that sporangia had been formed in the flasks on the desk, that the mycelium in the flask subject to frequent shaking showed no sign of sporangia, but consisted of balls of hyphae, the whole very dense and vigorous, and that on the mycelium in the flasks subject to a slight jar there were many aborted sporangia (fig. 1). It was evident that in the latter case a condition of starvation had momentarily existed, stimulating the ends of the hyphae to the production of sporangia; that before these could fully develop, the slight jar of the liquid had resulted in a fresh supply of food being brought to the hyphae, resulting in renewed vegetative growth and the abortion of the partially formed sporangia. The formation of sporangia in the pea agar is doubtless to be accounted for by the slow diffusion of food in the thick medium. The fact that only some species behave in this way doubtless indicates a more vigorous metabolism on the part of such species; the rate of metabolism exceeds the rate of diffusion and the result is starvation.

Saprolegnia Kaufmanniana has also proved interesting in that it shows great sensitiveness to the concentration of haemoglobin in solutions into which vigorous mycelia are placed. While such forms as *S. ferax*, *S. mixta*, or *S. monoica* will produce oogonia more or less freely in concentrations of haemoglobin varying from 0.075 to 0.01 per cent, *S. Kaufmanniana* persistently refused to respond to any concentration except 0.025 per cent. In this, either alone or with certain salts, oogonia containing oospores and accompanied by antheridia were regularly produced, though never in large numbers.

Achlya Klebsiana, n. sp.

This species was collected under three numbers, the plants all proving similar, and all were isolated as single spore cultures during November and December 1913. The cultures were secured from collections of algae from Bass Lake, near Ann Arbor, Michigan, and from near Coldwater, Michigan, in both cases collected by Mr. E. B. Mains. One culture was also secured from a dish of algae in the botanical laboratories of the University of Michigan, of unknown source, but doubtless from around Ann Arbor.

Hyphae stiff, medium thick, forming a dense zone about the fly. Among these are large, coarse, branched hyphae attaining a length of 10–15 mm. or sometimes more; sporangia cylindrical, discharging and forming secondary sporangia as in *A. prolifera*, but developing in pea agar or in pea extract when this is absolutely quiet; oogonia on short lateral branches which are about as long as the diameter of the oogonia, rarely at the ends of long hyphae, but nearly always on the basal portions of strong hyphae, near the body of the fly, never intercalary; round or slightly oval in shape; oogonium wall smooth, not pitted, oospores 4–10, averaging about 25 μ in diameter, excentric; antheridia always present, of diclinous origin, partly clasping the oogonia, never clavate nor wrapped about the oogonia; gemmae produced in chains by the breaking up of the large hyphae, cylindrical, sometimes slightly branched or with one or more protuberances at one or both ends.

This species is peculiar in the fact that besides the zone of delicate hyphae which usually surrounds the fly, there were also a number of very long, thick hyphae. These commonly extended for several millimeters beyond the thick tuft of hyphae and spread out on the surface of the liquid, later becoming densely filled with protoplasm and breaking up into chains of gemmae as shown in fig. 2.

Oogonia are produced quite regularly on flies and are always clustered near the body of the fly, but so far, with one exception, I have been unable to secure oogonia in artificial media. In one test a sterilized pea on which the fungus was growing was left in an open dish of distilled water. Bacterial decay set in slowly, the water was changed from time to time, and the fungus kept on growing vigorously, eventually forming oogonia. In no solution of haemoglobin or leucin, with or without salts or sugars, have oogonia with oospores appeared, though empty oogonia have been occasionally formed in haemoglobin. In one case penetration of the oogonium was observed (fig. 3), but whether fertilization takes place is not known. This species shows affinities with *A. DeBaryana* Humphrey in the excentric oospores and smooth unpitted oogonia, but the strictly diclinous, branched antheridia and the arrangement of the oogonia distinguish it markedly from that species. In *A. DeBaryana* the oogonia are arranged in a loose raceme along the hyphae well out from the fly, while in *A. Klebsiana* the oogonia are always borne in a dense cluster near the body of the fly.

Saprolegnia Kaufmanniana, n. sp.

This species was collected from algal material in the botanical laboratory of the University of Michigan, of unknown source, but presumably from around Ann Arbor.

Vegetative growth like that of *S. ferax*, with firm stiff hyphae; sporangia freely produced and of the same size and appearance as in *S. ferax;* gemmae round, oval, or irregular in shape, mostly single, sometimes in chains and freely produced; oogonia very large, on long or short stalks, or intercalary, scattered; oval or club-shaped, very rarely almost round, the usual size being about 70–80 $\mu\times$100–250 μ. The smallest oogonium noted was 30$\times$70 μ; oogonium wall thin and smooth, without pits; oospores from 3 or 4 in small oogonia to very many in large ones, averaging about 20–30 oospores per oogonium; oospores average about 30 μ in diameter, contents granular without any conspicuous oil drop; antheridia nearly always present, only occasionally absent on intercalary oogonia, diclinous, of various shapes from clavate to clasping or irregular, often curving part way round the oogonium, and borne on slender antheridial branches; usually more than one on an oogonium.

This species seems to differ decidedly from all others described, especially in the large, thin-walled oogonia without pits. Rarely two oogonia were observed in series, as in fig. 5. This species may be related to *S. anisospora*, of which species little is known, though no evidence of two kinds of zoospores was found in the present species. Besides its marked morphological characters, *S. Kaufmanniana* is interesting from the fact that it is especially sensitive to the concentration of haemoglobin. Oogonia were but sparingly produced on flies, many cultures having none, and no culture having more than a few. Tests were made by transferring vigorous mycelium to haemoglobin solution, and it was found that only where the haemoglobin had a concentration of 0.025 per cent were oogonia formed. Of the drawings, fig. 7 is from fly cultures, the others from haemoglobin 0.025 per cent.

S. MONOICA var. **vexans**, n. var.

This was secured from algal material collected at Sukey Lake, near Ann Arbor, Michigan. The vegetative growth, sporangial characters, and the formation and shape of gemmae do not differ in any particular from those present in *S. monoica*, *S. ferax*, or any other species of that group except *S. mixta*, which has weaker hyphae. The material was cultivated for nearly a year and a half on flies, in agar, and by transfer from a strong culture medium such as pea decoction or peptone, into haemoglobin, leucin, peptone, or other solution. During all this time no oogonia were produced. Toward the end of this time a series of tests was being made with several cultures by transferring vigorous mycelium to leucin to which various sugars and salts had been added. Among other combinations there was used leucin $\frac{M}{200}$ + levulose $\frac{M}{200}$, and in this a mycelium out of pea extract produced an abundance of oogonia. When these were examined they proved to be indistinguishable from the oogonia and antheridia of *S. monoica* Prings. Rarely an oogonium was found on which there was no antheridium, but in some solutions this may also be the case with *S. monoica*.

The fact that cultures of *S. monoica* were going on at the same time suggested the possibility of contamination. Check cultures were made, therefore, by taking mycelium from the dish in which the oogonia were formed and growing this on fly. Had the mycelium producing oogonia been that of *S. monoica* (no. 79*c* of my series), plenty of oogonia would have been produced. In fact, no oogonia were formed on the fly culture, but a fresh culture from this fly through pea decoction into leucin and levulose again produced oogonia as before.

We seem to have here, therefore, the remarkable case of a variety of *S. monoica* having lost sexuality, but recovering it under stimulus of this special combination, leucin and levulose in concentration $\frac{M}{200}$ each.

The gemmae of this form are perhaps a little more varied in shape than is the case with the species, but the shape of these organs is so variable in most species that they are of no value for systematic purposes.

Had time permitted, it would have been interesting to cultivate this form for many generations in leucin-levulose solutions to determine whether the vigorous production of oogonia which characterizes such forms of *S. monoica* as my 79*c* would be regained by this variety.

The forms described in this paper are remarkable examples of the intimate dependence of the members of this group on external conditions.

UNIVERSITY OF MICHIGAN

EXPLANATION OF PLATE XXI

FIGS. 1–4.—*Achlya Klebsiana*.

FIG. 1.—Portions of hyphae showing aborted sporangia; tip of one (at *a*) has also died and would shortly have been pushed aside; about ×85.

FIG. 2.—Short chain of gemmae showing how they break away and fall off; about ×85.

FIG. 3.—Oogonium with oospores; ×300.

FIG. 4.—Young oogonium showing much branched antheridial hyphae; ×300.

FIGS. 5–7.—*Saprolegnia Kaufmanniana*.

FIG. 5.—Two oogonia in series; ×300.

FIG. 6.—Intercalary oogonium; ×300.

FIG. 7.—Oogonium showing several antheridia; ×300.